Neuro-Oncology
23(1), 23–33, 2021 | doi:10.1093/neuonc/noaa158 | Advance Access date 5 July 2020

# Synaptic input to brain tumors: clinical implications

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**Abstract**

The recent discovery of synaptic connections between neurons and brain tumor cells fundamentally challenges our understanding of gliomas and brain metastases and shows how these tumors can integrate into complex neuronal circuits. Here, we provide an overview of glutamatergic neuron-to-brain tumor synaptic communication (NBTSC) and explore novel therapeutic avenues. First, we summarize current concepts of direct synaptic interactions between presynaptic neurons and postsynaptic glioma cells, and indirect perisynaptic input to metastatic breast cancer cells. We explain how these novel structures drive brain tumor growth and invasion. Second, a vicious cycle of enhanced neuronal activity, including tumor-related epilepsy, and glioma progression is described. Finally, we discuss which future avenues to target NBTSC appear most promising. All in all, further characterization of NBTSC and the exploration of NBTSC-inhibiting therapies have the potential to reveal critical vulnerabilities of yet incurable brain tumors.

## Key Points

1. Comprehensive overview of glutamatergic neuron-to-brain tumor communication is provided.
2. Clinical implications for brain tumor–related epilepsy and potential therapeutic avenues are discussed.

Gliomas and brain metastases remain formidable therapeutic challenges in neuro-oncology.[1,2] Despite decades of extensive research, the exact pathophysiological mechanisms for their inevitable progression remain incompletely understood. A close relationship between the nervous system and cancer is crucial for the initiation and progression of various brain tumors, which is supported by ample data from the rapidly emerging field of "cancer neuroscience."[3–8] Along these lines, it was recently demonstrated that neurodevelopmental pathways are exploited by glioma cells themselves, which contributes to brain tumor progression and resistance.[9–11] Cells of incurable gliomas extend long, thin membrane tubes, called tumor microtubes, which share features with axonal and dendritic outgrowth of developing neurons.[9,10] In a separate line of research, it has been shown that neuronal activity can drive glioma progression via paracrine mechanisms—for instance, by secretion of neuroligin-3, which in turn induces a synaptic gene signature in glioma cells.[4,5] Even tumors outside the brain exploit neuronal communication mechanisms, which include glutamatergic, cholinergic, and adrenergic signaling and promote or inhibit tumor growth.[12–14] Ultimately, all these lines of research culminated in the recent discovery of glutamatergic communication between neurons and brain tumor cells that drive brain tumor progression in high-grade pediatric[15] and adult gliomas[16] and breast cancer brain metastases.[17] It has to be noted that both direct synapses and indirect perisynaptic contacts reminiscent of tripartite synapses[18] were found on glioma

cells, whereas metastatic cells exhibited perisynaptic contacts only.[17] Although these fundamental differences exist, we propose here to unite these two concepts because both highlight the intricate communication of neurons and brain tumor cells. We therefore chose the term "neuron-to brain tumor synaptic communication" (NBTSC), which covers both direct and indirect synaptic communication.

Our increasing understanding of neuron–brain tumor cell interactions is paralleled by a growing field of research focusing on the intimate interplay of neurons with neural precursor cells (NPCs) and oligodendrocyte precursor cells (OPCs). Bergles et al demonstrated in 2000 that neurons form bona fide synapses onto OPCs,[19] describing for the first time a neuron-to-non-neuron synapse. These neuroglial synapses on OPCs seem to have a regulatory influence on proliferation and differentiation.[20,21] However, the exact functions of neuron-OPC synapses are still not fully understood, even though extensively debated.[22] Thus, parallel study of synapses in development and malignant disease could offer further insights into the role of neuron-to-non-neuron synapses.[22] Recent evidence suggests that the cells of origin in gliomas are NPCs and/or OPCs.[23,24] In accordance with this, a recent study proposed that glioma cells exist in one of 4 different states: NPC-like, OPC-like, astrocyte-like, and mesenchymal-like, with varying proportions of cellular states in adult and pediatric glioblastoma in tumor samples of the same entity and a depleted astrocyte-like state in pediatric glioblastoma.[25] It was also demonstrated that cellular states with an NPC- and OPC-like expression pattern contain the highest fraction of proliferating cells, especially in pediatric gliomas,[25] another layer of heterogeneity that demands attention when targeting synaptic input onto glioma cells. It is therefore a very interesting question whether the putative descendance from and the genetic similarities to these precursor cells reveal new insights into how mechanisms of neuronal communication are exploited by brain tumor cells: to promote their proliferation, but also for the first steps of tumorigenesis. For NBTSC, an enrichment of synaptic genes was shown in OPC-like tumor cells in a pediatric glioma entity.[15]

In this perspective article, we aim to provide a conceptual framework of NBTSC for neuro-oncologists: its main features, its relevance for a vicious cycle between brain tumor growth and epilepsy, and, ultimately, how it might be targeted pharmaceutically as a novel antitumor therapy.

## The Discovery of Synaptic Structures Between Neurons and Brain Tumor Cells

By using electron microscopy, distinct synapses formed by glioma cells were identified that possess all hallmarks of neuron-to-neuron synapses (Fig. 1A).[15,16] These included

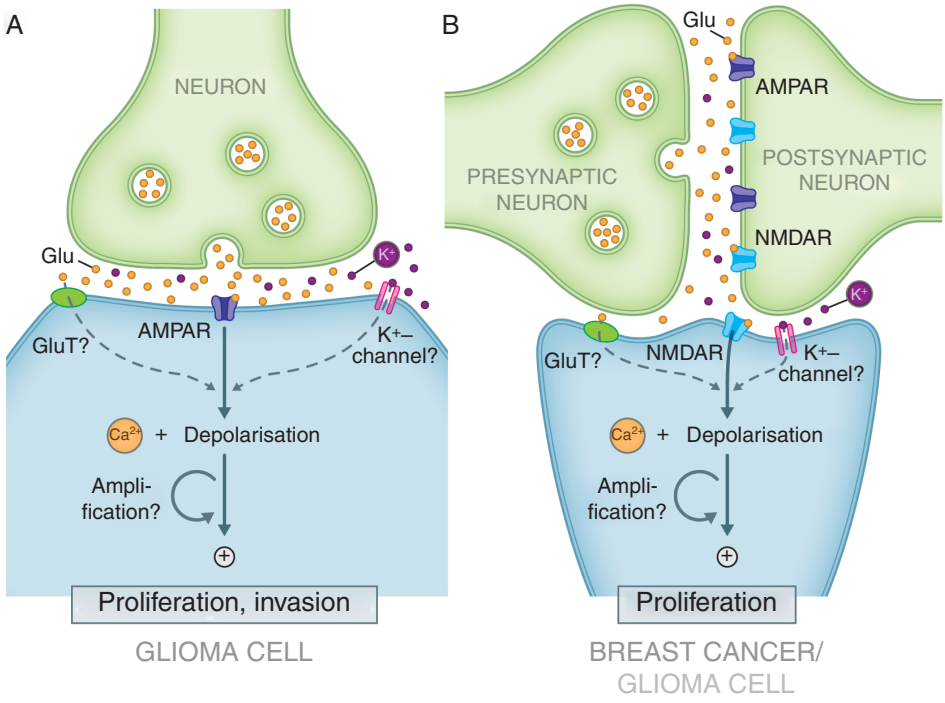


**Fig. 1** Current knowledge of direct (A) and indirect (B) neuron-to brain tumor synaptic communication (NBTSC). Direct, bona fide synapses have so far only been found in glioma (A), whereas indirect, perisynaptic interactions have been demonstrated structurally and functionally for brain metastases of breast cancer, and structurally for glioma (B). AMPARs mediate synaptic transmission of synaptic contacts on glioma, and genetic and pharmacological perturbation leads to reduced proliferation and invasion. In breast cancer brain metastases, perisynaptically located NMDARs mediate the growth of brain macrometastases. Potassium (K+) channels and glutamate transporters (GluT) have been shown to partially mediate neuron-mediated currents, at least in glioma cells. Their exact role and location are still unclear.

diffuse and anaplastic astrocytomas, glioblastomas, and diffuse intrinsic pontine gliomas,[15,16] which are all incurable brain tumor types. In breast cancer brain metastases, the malignant cells approach neuron-to-neuron synapses of the brain in a perisynaptic fashion,[17] resembling the tripartite configuration of neurons and astrocytes[18] (Fig. 1B). Interestingly, such tripartite synaptic configurations were also found in adult glioma, in addition to direct synaptic contacts.[16] However, the specific function of perisynaptic contacts for adult glioma remains unknown. Importantly, most synapses were found in the infiltration zone of gliomas,[16] where neurons are still viable. Diffuse infiltration of the central nervous system is an important pathological hallmark of gliomas which renders the disease incurable.[26]

To examine the functionality and properties of these synaptic structures, patch-clamp recordings of single tumor cells were performed. With this sensitive electrophysiological approach, currents flowing across the cell membrane can be precisely measured. In glioma cells, 2 types of depolarizing currents were found[15,16]: (i) fast excitatory postsynaptic currents (EPSCs) and (ii) prolonged slow inward currents (SICs). EPSCs are seen in neurons, as well as OPCs,[19] and can be mediated by different neurotransmitter receptors, with the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), a member of the ionotropic glutamate receptor family, being the most common. Indeed, glioma cell EPSCs have kinetics that are similar to AMPAR-mediated EPSCs and could be blocked with AMPAR antagonists. Further experiments suggested that AMPARs in glioma cells are permeable to calcium ions.[27] These calcium-permeable AMPARs ($AMPAR^{Ca}$) are found in some specialized synapses, on OPCs and other glia. Of note, neuron-to-glioma synapses exist, both structurally and functionally, also in freshly resected human material of low-grade and high-grade astrocytoma, including glioblastoma.[16] In addition to fast EPSCs lasting for only a few milliseconds, both pediatric and adult glioma cells exhibited SICs, which last over several tens of milliseconds to seconds. The nature of these currents is not entirely understood but is driven by neuronal activity. In pediatric glioma cells, SICs are predominantly mediated by potassium channels.[15] Here, extracellular potassium accumulates during strong neuronal activity and thereby depolarizes the surrounding glioma cells, reminiscent of long-lasting depolarizations elicited by potassium in astrocytes.[28] As far as we know, these currents can be mediated by a variety of channels in adult gliomas, including AMPARs, glutamate transporters, and potassium channels.[16] At this moment in time it is not clear whether SICs arise from synaptic or perisynaptic contacts in adult gliomas (Fig. 1). Moreover, it is unresolved whether discrepancies in pediatric and adult gliomas are due to methodological differences of the recordings or reflect true biological differences. In breast cancer brain metastases, N-methyl-D-aspartate receptors (NMDARs), another subtype of ionotropic glutamate receptor, were located close to neuron-to-neuron synaptic clefts, and were identified as a driver for metastatic progression (Fig. 1B). Physiologically, NMDAR are located postsynaptically in neurons and are associated with synaptic plasticity in memory and learning. However, in the pseudo-tripartite configuration, NMDARs are also located in the vicinity of the synaptic cleft within the plasma membrane of breast cancer cells. This way the high affinity NMDAR can plausibly be activated by glutamate that spills out of the synaptic cleft and potentially leads to $Ca^{2+}$ inflow and depolarization in breast cancer cells (Fig. 1B).

All functionally characterized synaptic proteins in glioma, including a particularly robust expression of AMPAR subunits, could also be validated molecularly with single-cell RNA-sequencing studies of existing databases from patient material of resected human gliomas.[15,16] Of note, not all glioma cells express synaptic genes, which is in line with the known heterogeneity of glioma cells.[9,10,25,29,30] This is also evident in the structural and functional data, which together allow the conclusion that only approximately 10–30% of glioma cells receive synaptic input. In breast cancer, gene expression analysis hinted at a particular role for NMDAR, particularly in the prognostically unfavorable basal-like/triple-negative subtype of breast cancer, and this was also correlated with reduced survival in patients.[17]

## NBTSC Drives Brain Tumor Progression

To further define the functional determinants of NBTSC, calcium imaging experiments were performed.[15–17] This method allowed short calcium transients to be correlated with depolarizations of the tumor cell membrane, suggesting that synaptic input is converted into a calcium signal that appears to be amplified in the tumor cell and that finally may activate downstream pathways (Fig. 1). Future research will elucidate the exact downstream mechanisms that are involved in the translation of synaptic activation to biological effects (eg, proliferation and invasion) that were observed in brain tumor cells.

Finally, a striking effect of NBTSC on tumor biology was described.[15–17] Genetic or pharmacological perturbation of glutamatergic neuron-to-glioma synapses impaired invasion of glioma cells and slowed proliferation, both in pediatric and in adult mouse models of incurable gliomas.[15,16] Furthermore, genetic perturbation of AMPAR significantly reduces migration velocity and glioma growth and increases survival of glioma-bearing mice.[15,16] Likewise, tumor cell density as observed through a cranial window in glioma-bearing mice in 2 different patient-derived glioblastoma cell lines over a 14-day period remained considerably stable in mice treated with the FDA-approved AMPAR antagonist perampanel, whereas it almost doubled in control mice.[16] In addition, the proliferation index of xenografted diffuse intrinsic pontine glioma cells was reduced by nearly 50%.[15] For breast cancer brain metastases, silencing of the NMDAR subunit *GRIN2B* (glutamate receptor inotropic N-methyl D-apartate 2B) decreased brain metastatic burden and prolonged survival in mice.[17] Here, NBTSC seemed to play a role for the later stages of brain metastases growth, but not so much for the initial (microscopic) seeding events. In contrast, in glioma, NBTSC seems especially relevant for more initial stages of tumor progression: diffuse brain colonization.[26] It will be interesting to learn whether the early stages of brain metastatic colonization are driven by other synaptic configurations, as well as whether NBTSC can be found across different cancer entities. While gamma-aminobutyric acid ($GABA_A$) receptor expression was upregulated by brain metastatic breast cancer cells,[7] a metabolic uptake

of GABA was implicated in the proliferative role of this neurotransmitter.[7] It remains to be investigated which role NBTSC might play in this context.

## Interplay of Epilepsy and Glioma Progression

In the previous paragraphs we have argued that neuronal activity promotes glioma progression via various direct and indirect mechanisms. Vice versa, it has become evident in recent years that glioma progression can in turn lead to increased neuronal activity due to neuronal hyperexcitability, the pathophysiological hallmark of epilepsy and a clinical complication occurring in 40–80% of glioma patients.[31,32] Therefore, we propose a fatal vicious cycle with the 2 cornerstones of neuronal hyperexcitability and glioma progression, which reinforce and amplify each other (Fig. 2).

Neuronal activity promotes glioma progression with glioma cell proliferation and potentially invasion by glutamatergic NBTSC. Additionally, neuronal activity has been shown to stimulate glioma cells via secretion of soluble neuroligin-3. The metalloprotease ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) sheds neuroligin-3 in a neuronal activity–dependent manner.[5] Furthermore, other soluble factors such as brain-derived neurotrophic factor (BDNF) and 78 kDa glucose-regulated protein (GRP78) are released upon neuronal activity and promote glioma cell proliferation.[4] Taken together, neuronal activity drives glioma progression via at least 2 routes: NBTSC and paracrine stimulation (Fig. 2).

Glioma progression in turn can promote neuronal hyperexcitability by various mechanisms. Brain tumor growth includes mass effects, tumor-surrounding edema, and diffuse infiltration of the brain parenchyma with disturbance of neurovascular coupling and normal neuronal networks.[33–35] Clinical data showing the effectiveness of antitumor therapies, including surgery, radio- and chemotherapy, and steroids in the treatment of tumor-related epilepsy, support the idea of a mechanistic link between brain tumor growth on one hand, and tumor-related epilepsy on the other.[36–38] This concept is also supported by the opposite observation: that worsening epilepsy is correlated with tumor progression.[39,40]

Another molecular mechanism adding to neuronal hyperexcitability is glutamate secretion by glioma cells, which is also known to promote glioma cell proliferation via auto- and paracrine mechanisms.[41,42] The pathway of glutamate secretion is mediated by the xc – cystine-glutamate transporter system, which can be inhibited by the FDA-approved drug sulfasalazine.[41,43] This could be a glioma-specific way of reducing neuronal hyperexcitability and glioma growth at the same time.[44] Additionally, glioma cells can boost hyperexcitability by inducing neuronal synaptogenesis,[45] and the fraction of glioma cells capable of this evolves in the course of glioma progression. Interestingly, this synaptogenesis could also be related to the onset of clinical seizure activity.[45] Recently, it has been demonstrated that seizure onset and incidence can be driven by glioma-specific mutations, particularly in the enzyme PIK3CA (phosphatidylinositol-4,5-bisphosphate

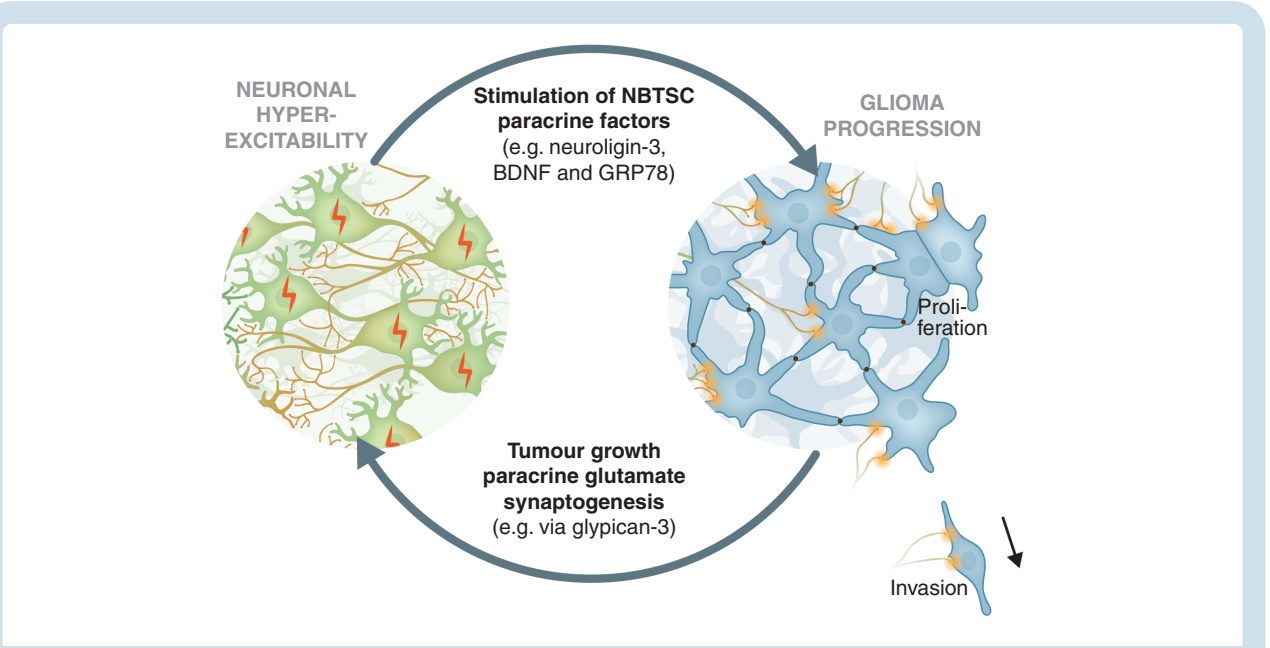


**Fig. 2** Concept for a vicious cycle between neuronal hyperexcitability, as seen in epilepsy, and glioma growth. Mechanisms of bidirectional communication leading to neuronal hyperexcitability and glioma cell proliferation/invasion are shown. Neuronal activity leads to direct stimulation of NBTSC as well as stimulation of glioma cells via paracrine factors such as neuroligin-3, BDNF, and GRP78. Glioma progression can in turn mediate neuronal hyperexcitability via tumor growth which includes its mass effect, the tumor edema, and diffuse infiltration of the whole brain disrupting healthy neuronal networks. Other molecular mechanisms such as secreted paracrine glutamate and aberrant synaptogenesis have been described as alternative mechanisms mediating neuronal hyperexcitability.

3-kinase catalytic subunit alpha), a component of the receptor tyrosine kinase (RTK)–Ras–phosphatidylinositol-3 kinase (PI3K) signaling pathway in human glioblastomas. Secreted glypican-3 was identified as a novel driver for induction of synaptogenesis and neuronal hyperexcitability.[46]

The positive feedback loop of neuronal activity and glioma progression (Fig. 2) suggests therapeutic opportunities to disrupt this vicious cycle. Although anti-epileptic drugs that act on the presynaptic side of neurons did not show a clear effect on overall survival of patients,[47] a beneficial effect might depend on the level of neuronal activity in individual patients and therefore might be dose and drug dependent. Furthermore, it is not clear whether these pre-synaptically acting anti-epileptic drugs that are most commonly used in the clinic today (levetiracetam, valproic acid, sodium channel inhibitors) reduce peritumoral aberrant neuronal function to an extent that an effect on tumor growth can be expected. Importantly, recent studies have shown that increased neuronal activity in glioma patients (although many patients were already treated with anti-epileptic drugs) appear to be a potential predictive marker for worse prognosis, and can be measured in principle with magnetoencephalography (MEG) or EEG.[34,48,49]

Some studies find that clinical presentation with epilepsy is a positive prognostic factor,[50,51] which is an apparent paradox: prognostically favorable low-grade brain tumors appear to be especially epileptogenic. It is clear today that those gliomas arise in young age and slowly grow over many years, sometimes decades before they are diagnosed.[52,53] Thus, such a tumor can influence surrounding neurons for years, and it is very likely that such a tumor will cause an epileptic seizure at some point in time during that extended growth period, with an epileptic seizure becoming the first clinical sign of the disease. Moreover, also subclinical neuronal hyperexcitability might play an underappreciated role in the progress of gliomas as most studies investigated only clinically apparent seizures. Taken together, the suggested relation of epilepsy and a good prognosis has to be taken with great caution in neuro-oncology. It would be necessary to correct for the frequency (not the overall occurrence) of epileptic seizures, including the amount of neuronal hyperexcitability, the total time of tumor growth in the brain, and the type of brain invasion and specific brain interactions in different tumor entities.

It remains an important task to better understand how much such a vicious cycle between epilepsy and glioma growth contributes to tumor growth and invasion in the human disease. Clinical imaging of tumor progression with simultaneous longitudinal electrophysiological monitoring of neuronal activity would clearly extend our understanding of this vicious cycle in patients. Lastly, it is an open question whether this vicious cycle is also in play in brain metastasis, where epilepsy is a relevant clinical problem, too.

## Development of Therapies Targeting NBTSC: Potential Avenues

When considering the described properties and functions of NBTSC, multiple avenues arise that can exploit these features for novel antitumor therapies (Figure 3). Current brain tumor treatments are either symptomatic or aim at the reduction of the tumor mass and not interactions of brain tumor cells with their surrounding tissue. In principle, targeting the progression-promoting, direct synaptic interference of neurons with brain tumor cells could open the way for a plethora of specific neuroactive drugs into neuro-oncology. Thus, in the following section we systematically discuss 5 possible approaches to target NBTSC.

### *Inhibition of synaptic and perisynaptic signal transmission*

In the previous paragraphs we have argued how glutamatergic NBTSC contributes to tumor progression. These effects could be reduced by interfering with AMPAR. Since the 1980s, great effort has been put into the development of competitive and non-competitive AMPAR inhibitors.[54] As early competitive AMPAR inhibitors exhibited unfavorable pharmacological properties,[55] the focus has shifted to non-competitive AMPAR inhibitors. So far, only perampanel has found its way into the clinic. It has been approved by the FDA for the treatment of partial-onset seizures. In contrast to the other AMPAR inhibitors to be discussed, it exhibited favorable pharmacokinetics in humans and an acceptable safety profile in 3 phase III trials in partial-onset seizures, with patients reporting mostly mild adverse effects, such as dizziness, somnolence, headache, fatigue, and irritability.[56] In glioma therapy, perampanel is hitherto used as an alternative or adjunctive therapy to treatment-resistant tumor-related epilepsy. A few small studies have reported good seizure control in glioma patients.[57–60] However, no systematic trial has investigated the effects of perampanel on tumor progression and survival of patients so far.

Talampanel, another non-competitive AMPAR inhibitor, has shown promising results in early trials in epilepsy,[61] but the development of the drug was terminated due to the short pharmacological half-life of 3 hours, necessitating multiple doses per day.[62] However, 2 single-arm phase II clinical trials were conducted in glioma with talampanel before its discontinuation. In one study talampanel monotherapy was tested in recurrent glioblastoma and anaplastic glioma. In the unselected patient cohort ($n = 32$) no significant activity of the drug was observed compared with historical controls.[63] In a larger study in primary glioblastoma, the combination of standard radiochemotherapy with talampanel showed promising overall survival results (primary endpoint) without additional toxicity compared with the standard of care.[64] As a matter of caution, only historical controls have been used as comparators in these trials.

All in all, a randomized placebo-controlled trial testing an AMPAR antagonist with a suitable pharmacokinetic profile such as perampanel that targets NBTSC in the tumor infiltration zone, in combination with standard of care (resection, radio- and chemotherapy) that effectively treats the main tumor mass, appears the most promising concept to assess the efficacy of AMPAR inhibition in gliomas.

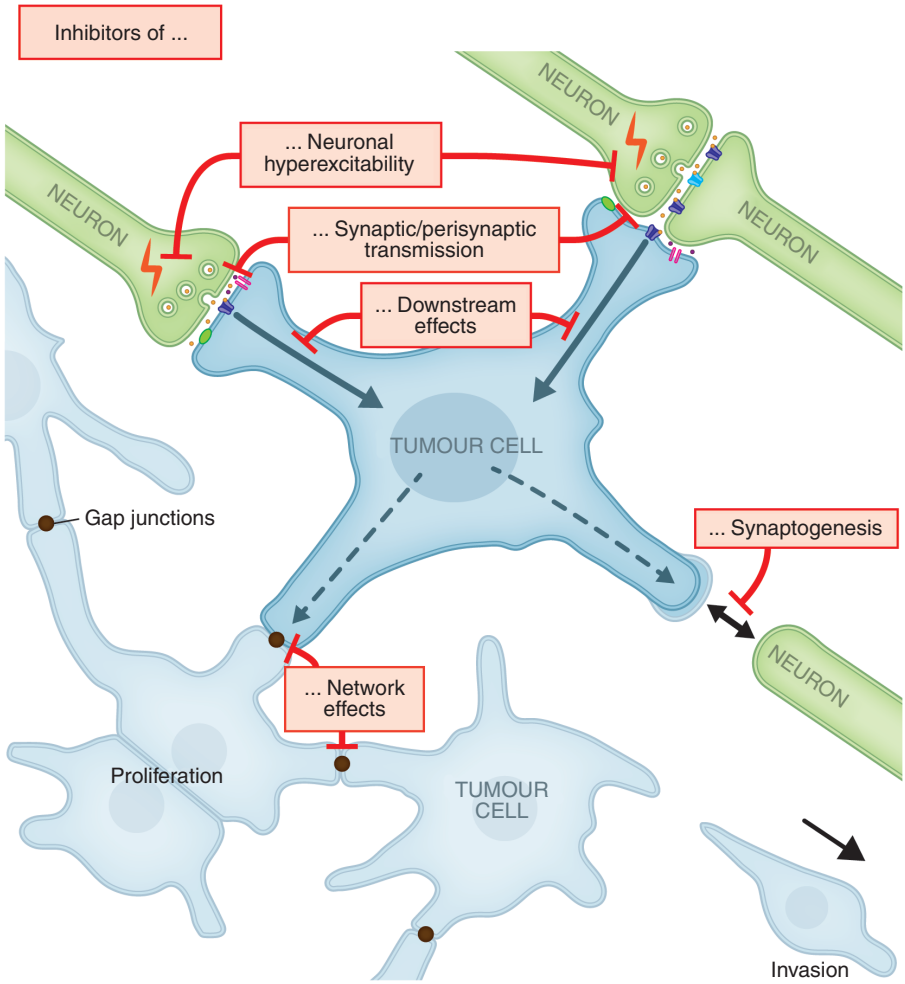


**Fig. 3** Potential therapeutic avenues for targeting NBTSC. Synaptic and perisynaptic interactions of neurons and brain tumor cells as well as downstream effects within tumor cells and across tumor networks are schematically depicted. Key mechanisms of NBTSC that can in principle be therapeutically targeted are shown in red boxes. For details, see text.

Furthermore, it is worth noting that at least a portion of AMPARs in neuron-to-glioma synapses are calcium permeable.[15,16] AMPARs are composed from a set of 4 subunits, GluA1 through GluA4, and their permeability to calcium depends on their subunit composition.[27] Only AMPARs either lacking the GluA2 unit or whose GluA2 subunit has undergone a specific co-transcriptional modification are calcium permeable. Most AMPARs are impermeable to calcium in the adult brain.[65] Calcium permeable AMPA receptors (AMPARCa) play a role in developing neurons, but in the adult brain, its expression is limited.[65–67]Therefore, specific inhibitors of AMPAR$^{Ca}$ could more specifically inhibit NBTSC, as opposed to general AMPAR inhibitors, and are thus promising candidates for the developments of novel glioma therapies. Interestingly, the AMPAR$^{Ca}$ inhibitor IEM-1460 displays central effects after systemic administration in mouse experiments[68] and could therefore be investigated for systemic glioma therapy. However, long-term therapeutic as well as adverse effects in animals and humans have to be assessed in future studies.

Taken together, AMPAR inhibition, particularly the inhibition of AMPAR$^{Ca}$, which should have a particularly high therapeutic window, emerges as a promising principle for glioma therapy that, however, needs to be validated in a properly controlled clinical trial (Fig. 3).

In addition to AMPAR, other neurotransmitter receptors may also play a role in NBTSC. NMDARs are involved in perisynaptic contacts of breast cancer metastatic cells.[17] However, targeting NMDAR comes with considerable challenges. Many pharmaceutical approaches to target NMDAR in the brain appear to have a small therapeutic window in humans and can cause serious adverse effects, ranging from psychiatric syndromes[69] to coma, as seen in autoantibody-mediated anti-NMDAR encephalitis.[70] Still, the non-competitive NMDAR antagonist memantine is clinically used for the treatment of Alzheimer's disease and could be explored in the treatment of breast cancer brain metastases—but its NMDAR inhibitory effects are mild.[71] Furthermore, recent advances in allosteric modulators of NMDAR[72]

could provide new drugs that could be tested in controlled trials of brain tumor therapies.

Apart from fast postsynaptic currents, we demonstrated that slow inward currents are evoked by neuronal activation. Although the exact mechanisms are not fully understood and their biological role remains obscure, the following channels and transporters are currently thought to be involved: potassium channels, glutamate transporter, as well as AMPARs. Targeting AMPARs was already discussed above, but potassium channels and glutamate transporters, besides AMPAR, may also be interesting targets (Fig. 3). The potassium-channel superfamily is diverse, and dysregulation in glioma has been linked to proliferation and invasion.[73] Many specific potassium channel blockers and modulators have been developed[74]; hence, it will be necessary to understand which potassium channel subtypes are potentially involved in slow current formation. Inwardly rectifying potassium (Kir) 4.1 has been shown to play a role in neuronal activity–dependent potassium currents in astrocytes.[28] While there is no specific inhibitor for Kir 4.1, tricyclic antidepressants and selective serotonin reuptake inhibitors can also inhibit Kir 4.1 and might be of interest for further investigation[74] (Fig. 3).

Glutamate transporters (GluTs) control the concentration of glutamate in and around the synaptic cleft, and different subtypes are found either pre-, post-, or perisynaptically in glial cells.[75] In gliomas, glutamate transport has been predominantly attributed to $x_c^-$ cystine glutamate exchanger, whereas classical GluTs are downregulated.[42] In vitro studies even hint at glioma-inhibiting effects by overexpression of GluTs.[76] These findings have to be reevaluated with the new insights into the possible contribution of GluTs to glioma biology.[16] Several GluT inhibitor classes are available: UCPH101-like, TBOA (threo-beta-benzyloxyaspartate)-like, and dihydrokainate inhibitors,[77,78] and can be investigated in future studies (Fig. 3).

### *Inhibition of mechanisms downstream of NBTSC*

As indicated above, the downstream mechanisms after glioma cell membrane depolarizations, in the form of EPSCs or SICs, are not well understood. Experiments suggest that synaptic input can be translated into calcium transients, which in turn could activate downstream pathways. Different routes of translation are possible, such as depolarization, which could directly activate voltage gated calcium channels (VGCCs) and allow calcium influx, or small concentrations of calcium passing through $AMPAR^{Ca}$ could be amplified by calcium-induced calcium release (CICR) or inositol 1,4,5-triphosphate–induced calcium release (IICR). In vitro and in vivo studies indicate inhibiting effects of VGCC blockage on glioma cell proliferation.[79] Several inhibitors of VGCC are available[80]; the most interesting for NBTSC inhibition could be the approved drugs gabapentin and pregabalin, which have an anti-epileptic effect (see also section on synaptogenesis and hyperexcitability). Gabapentin and pregabalin act on the VGCC auxiliary subunit $\alpha 2\delta$ and modulate VGCC trafficking.[81–83] CICR and IICR play a crucial role in neurons[84] and glial cells,[85] and inhibitors such as ryanodine and thapsigargin are available.[86,87] Thus, it will be necessary to understand the exact downstream mechanisms of NBTSC that could serve as additional targets for glioma therapy (Fig. 3).

### *Disruption of electric coupling in glioma cell networks*

We have demonstrated before that glioma cells interconnect via gap junctions on tumor microtubes to a functional network, which renders gliomas more resistant to standard therapies.[9] As our current studies indicate, gap junctions may also couple glioma cells electrically. Gap junction blockers decreased the frequency or amplitude of SICs recorded in a single cell, respectively, and slowed down proliferation in the murine model.[15,16] Gap junction blockers are effective anticonvulsants in animal studies and thereby potentially reduce neuronal hyperexcitability and downstream network effects of NBTSC.[88] One interesting gap junction modulator is tonabersat, which has been investigated in the treatment of migraine and is generally well tolerated.[89] In a 2019 study, tonabersat was tested as an adjuvant therapy on a glioblastoma model in rats and it decreased tumor growth when used in combination with radiochemotherapy compared with radiochemotherapy alone.[90] Whether gap junction blockers such as tonabersat and meclofenamate can be used in human glioma therapy has to be elucidated by future research (Fig. 3).

### *Inhibition of neuron-to-glioma synaptogenesis*

Another possible avenue may be to prevent the formation of new malignant synapses in the first place. Synaptogenesis in the central nervous system is an incredibly complex process with many remaining open questions.[91–93] It involves the expression of pre- and postsynaptic proteins, the approximation of a presynaptic and a postsynaptic membrane, and subsequent synapse maturation. Synaptic contacts in neurons can be initiated by filopodia either from axonal or dendritic growth cones that sprout out in a seemingly random manner.[94,95] Once in approximation, the cell membranes are stabilized via adhesion molecules. The most prominent members, neurexins at the pre- and neuroligins at the postsynaptic site, have been shown to be sufficient to induce the opposing side to differentiate.[96,97] As described above, soluble neuroligin-3 can induce a synaptic gene signature in glioma cells. Therefore, as Venkatesh et al propose, either soluble neurexins or ADAM10 inhibitors[5] (ADAM10 is shedding neuroligin-3) could be a viable approach to decrease malignant synaptogenesis. Trial concepts regarding the latter are already on the way. Additionally, glial cells have been demonstrated to promote neuronal synaptogenesis by secreting stimulating molecules, such as thrombospondin.[98] Furthermore, gabapentin antagonizes thrombospondin binding to its receptor, the calcium channel auxiliary protein $\alpha 2\delta$, and thus effectively inhibits excitatory synaptogenesis.[81] Pregabalin has also been shown to bind to $\alpha 2\delta$, thus also potentially inhibiting synaptogenesis.[82] Whether gabapentin and pregabalin also exert these effects in experimental models of glioma and, most importantly, whether they show antitumor effects by NBTSC prevention are unknown and would be an interesting question for further preclinical research (Fig. 3). Since glypican-3 has been involved in the synaptogenesis

of certain glioma subtypes,[46] recent immunotherapeutic approaches as well as drugs targeting glypican-3 in liver cancer could be re-employed for distinct glioma types.[99,100] It will be interesting to see whether cell adhesion proteins (eg, neuroligin-1, neuroligin-2) as well as other molecules (eg, thrombospondin, tumor necrosis factor-α, Wnt) implicated in physiological synapse formation[92,93] play a role for glioma synaptogenesis and could therefore act as potential additional therapeutic targets.

### *Inhibition of neuronal hyperexcitability and subsequent brain tumor stimulation*

Neuronal hyperexcitability might be a main driver of glioma (Fig. 2) and hypothetically also of brain metastases progression, and therefore the general use and the specific selection of anti-epileptic drugs might be of profound clinical relevance. Measuring neuronal activity with EEG or MEG measurements is essential to evaluate the effectiveness of these therapies on subclinical neuronal hyperexcitability which might differ from patient to patient.[49,101] The role of anti-epileptic drugs working on the presynaptic side is unclear and needs to be investigated. The specific conceptual advantages of AMPAR inhibitors which act postsynaptically and inhibit not only seizure formation but also NBTSC in gliomas are evident. Additionally, gabapentin and pregabalin may have inhibiting effects on both VGCC and synaptogenesis, as well as anti-epileptic effects. Using the emerging knowledge about the mutual interdependency of neuronal hyperexcitability and brain tumor progression, targeted therapies with perampanel (or other selected anti-epileptics) could target both sides: reducing neuronal hyperexcitability, as well as inhibiting brain tumor stimulation by acting on NBTSC (Fig. 3). The effects of specific anti-epileptics have to be systematically assessed in future trials, potentially stratified for glioma subtypes.

The above-discussed 5 therapeutic avenues aim at disrupting the stimulatory neuronal input to brain tumor cells, but are not necessarily cytotoxic, more likely cytostatic.[15,16] Therefore, it needs to be evaluated whether or not synergistic effects exist between NBTSC inhibition and established cytotoxic treatment modalities such as radio- and chemotherapy—or whether anti-NBTSC therapies are better given as monotherapies (eg, after completion of standard therapies, or in the recurrent situation). Furthermore, the effect of neuromodulatory drugs on brain tumor cells has to be studied comprehensively as the inhibitory, GABAergic neurotransmitter system might inhibit glioma cell growth[102] as opposed to the excitatory, glutamatergic neurotransmitter systems.[15–17] Along the same line, the exact effects of these therapies on the tumor microenvironment have to be studied in disease-specific models.

Apart from developing therapies directed against NBTSC, it will also be important to measure responses to these therapies in gliomas. Current clinical imaging mainly captures the main tumor mass. However, since direct neuronal interactions play a particular role in the glioma infiltration zones,[15,16] it will be instrumental to develop clinical imaging paradigms that will be able to faithfully measure the glioma cell amount in infiltrated areas of the entire brain.

Lastly, brain tumors are anatomically, functionally, and molecularly heterogeneous diseases.[9,10,29,30] It will be important to understand how NBTSC contributes to the course of brain tumors in specific entities and possibly between individual patients, since, for instance, only a fraction of glioma cells receive synaptic input,[15,16] and it is likely that this fraction will be different between patients. It will therefore be important to define molecular and functional signatures of NBTSC: as predictive biomarkers and potentially also as biomarker read-out for the effectiveness of anti-NBTSC therapies.

## Conclusions and Outlook

In this review, we illustrate how the unexpected finding of synaptic communication between neurons and brain tumor cells is transforming our understanding of glioma and brain metastatic progression. Basic mechanisms of neurotransmission, including glutamatergic AMPAR and NMDAR signaling, are hijacked by malignant cells and promote tumor cell proliferation. Moreover, a mutual reinforcement with aberrant neuronal activity, as during epilepsy, further facilitates tumor growth. Since these communication pathways are not unique to tumor cells, it will be crucial to target mechanisms specific to NBTSC while preserving the functional integrity of the normal central nervous system. Pharmacological inhibition of AMPARs has already shown promising results, and further work will help to identify downstream targets of NBTSC, and their potential translational relevance. In addition to glutamate, extensive research has investigated the role of other neurotransmitters in brain tumor biology (reviewed in detail by Jung[3]), and the possibility that some effects may also be conveyed via NBTSC should be explored in future research. Furthermore, imaging parameters that better measure diffuse brain colonization, ideally also the activity of NBTSC, would greatly facilitate the development of therapies. In consequence, targeting NBTSC could specifically address the microscopic stages of brain tumor development that cannot be effectively controlled yet—and that continue to make gliomas and brain metastases incurable whole-brain diseases.

## Keywords

glioblastoma | DIPG | Brain metastases | Synapses | Cancer Neuroscience

## Funding

V.V. was supported by the Physician-Scientist program of the University of Heidelberg. W.W. and F.W. were supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 1389). T. Kuner was supported by the CellNetworks Excellence Cluster (EXC 81).

**Conflict of interest statement.** F.W. and W.W. are inventors of patent WO2017020982A1 "Agents for use in the treatment of glioma." This patent covers new treatment strategies that all target the formation and function of tumor microtubes in glioma. F.W. is co-founder of DC Europa Limited, and reports research collaborations with GlaxoSmithKline, Genentech, and Boehringer.

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